Research Article

SMEAR AND CULTURE EXAMINATION OF CLINICAL SAMPLES FROM SUSPECTED PATIENTS FOR *MYCOBACTERIUM TUBERCULOSIS*

Amit Kumar*[1], Ajay Kumar[2], Kanchan Sharma[2], Sandip Patil[1], Varun Chauhan[1], Amit Pratush[2], P.C. Sharma[1]
1. Shoolini University of Biotechnology and Management Sciences, Solan- 173212, India.
2. Shoolini Institute of Life Sciences and Business Management (SILB), Solan- 173212, India.
***Corresponding author's E-mail:** amit.thakur2035gmail.com

Accepted on: 17-11-2011; **Finalized on:** 20-01-2012.

ABSTRACT

Diagnosis is an important part to identify the causative organism and to help in prognosis of the disease. The clinical samples were collected from the suspected patients of tuberculosis during the course of the study. A total of 178 clinical samples were collected for the study including 38 samples of sputum, 30 of urine, pleural fluid (35), ascitic fluid (12), endometrial biopsy (20), CSF (14), pus (27) and pericardial fluid (2). The present study was aimed to do the smear and culture examination of clinical samples from suspected patients of *tuberculosis.* Among all the 178 samples collected, 18 samples were found smear positive and mostly were of sputum. All smear positive samples were also found positive on LJ growth medium.

Keywords: Clinical samples, Pus, Tuberculosis, *Mycobacterium tuberculosis*.

INTRODUCTION

Despite progress in the past decades, tuberculosis (TB) remains a major public health problem worldwide. About one-third of the world population is latently infected with *Mycobacterium tuberculosis,* and 10% of those infected persons develop disease during their lifetime[1]. India is contributing nearly one third of the world's Tuberculosis (TB) cases and has the highest rate of new TB cases[2]. The ability of *Mycobacterium tuberculosis* to adapt to different environments in the infected host is essential for its pathogenicity. Consequently, this organism must be able to modulate gene expression to respond to the changing conditions it encounters during infection[3]. The risk of tuberculosis (TB) in healthcare workers (HCWs) is related to its incidence in the general population and increased by the specific risk as a professional group[4]. The present study was done at SILB Solan in collaboration with IGMC (Indira Gandhi Medical College), Shimla Himachal Pradesh, India. In the present study 178 clinical samples were collected from suspected patients of tuberculosis. The major objective of the study was to help in the diagnosis of TB in suspected patients and so as to start treatment as soon as possible in those found to be infected with *M. tuberculosis.*

MATERIALS AND METHODS

Collection of clinical samples

Samples were collected from the suspected patients under asceptic conditions in a leak proof sterile container and were marked properly in order to avoid any mistake. Sputum, urine, pleural fluid, ascetic fluid, endometrial biopsy, CSF, pus and pericardial fluid (Table 1) were some of the important samples processed and formulated judiciously.[5]

Decontamination and Smear preparation

Decontamination of sputum with 4% NaOH is necessary in order to kill other microorganisms present and concentrate the bacilli only. All the specimens were centrifuged at 3000 rpm for 30 minutes. Then the supernatant was discarded and deposits were used for the preparation of smears. Smears were stained with an acid fast stain- Ziehl-Neelsen staining[5].

Isolation of *Mycobacterium tuberculosis*

After presumptive diagnosis, confirmation of suspected samples for *Mycobacterium tuberculosis* was done by culturing on suitable media. Collected samples were processed properly by centrifugation at 3000 RPM for 30 minutes. Supernatant was discarded and deposit remained was inoculated on the LJ media slants. All the slants were incubated at 37°C for 6-8 weeks till the appearance of growth. Further identification was done on the basis of colony morphology and smear examination[5].

RESULTS AND DISCUSSION

Diagnosis is the important part to identify the organism and to do the prognosis of the disease. The laboratory diagnosis of tuberculosis is based on microscopy or culture of the clinical samples. Although, these techniques either lack the sensitivity or are time consuming[6-7]. During the course of the study, cases of pulmonary tuberculosis were more prevalent than other type of TB. Samples from suspected patients were collected routinely and processed under asceptic condition in order to diagnose Tuberculosis. After years of decline, tuberculosis (TB) has re-emerged as a serious public health problem worldwide causing significant mortality and morbidity in developing countries[8].

The present study showed the epidemiological aspects of Tuberculosis in Himachal Pradesh. Various types of clinical

samples were collected for the study like 38 samples of sputum, 30 of urine, pleural fluid (35), ascetic fluid (12), endometrial biopsy (20), CSF (14), pus (27) and pericardial fluid. Out of 178 total processed samples *M. tuberculosis* was detected in 18 samples (Table 2). According to the WHO guidelines for TB control, patient with more than three weeks history, his cough should be screened for pulmonary tuberculosis through direct sputum smear examination for the presence of *M. tuberculosis*. Diagnosis of tuberculosis can be done by the demonstration of *M. tuberculosis* in clinical samples, because the clinical signs and symptoms of pulmonary tuberculosis are not so specific. So the examination of clinical samples for presence of *M.tuberculosis* is necessary before starting treatment[9].

Table 1: Distribution of all the collected samples

Samples	No. of samples
Sputum	38
Urine	30
Pleural fluid	35
Ascitic fluid	12
Endometrial biopsy	20
CSF	14
Pus	27
Pericardial fluid	2

Table 2: Distribution of number of positive and negative samples in smear examination by acid fast staining

Samples	No. of samples	Positive	Negative	Percentage of positive samples
Sputum	38	12	26	31.58%
Urine	30	2	28	6.7%
Pleural fluid	35	0	35	0%
Ascitic fluid	12	0	12	0%
Endometrial biopsy	20	0	20	0%
CSF	14	1	13	7.14%
Pus	27	3	24	11.11%
Pericardial fluid	2	0	2	0%

Table 3: Growth of suspected samples inoculated on LJ media

Samples	No. of Positive samples after smear examination	No. of Positive samples after culture on LJ medium	Percentage of positive samples
Sputum	12	12	100
Urine	2	2	100
CSF	1	1	100
Pus	3	3	100

Among 18 positive samples 12 were of sputum, 2 of urine, 1 of CSF and 3 of pus samples were found to be positive (Table-2). Smear examination was done with ZN staining which confirmed the presence of acid fast bacilli (Fig-1 & 2). On the basis of positive culture and smear, the patients were treated for TB by medical personnel. The suspected samples were also inoculated on LJ media for culture examination. All the 18 positive samples on the basis of smear examination (Table 3) were showed yellow colour colony growth on LJ media (Fig-3) confirmed the presence of *M. tuberculosis*. However, during early diagnosis of Tuberculosis other symptoms like fever, loss of weight, blood counts can be taken into consideration[9].

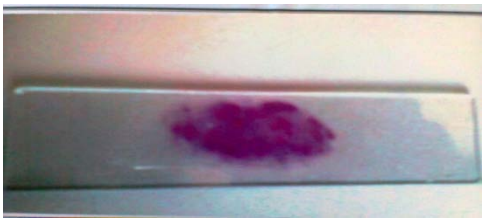

Figure 1: Slide showing Acid fast staining.

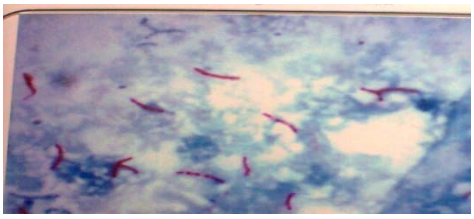

Figure 2: Acid fast bacilli after ZN staining.

Figure 3: Growth of *M.tuberculosis* on LJ media.

CONCLUSION

These tests help in early diagnosis of Tuberculosis because most often the disease remains undiagnosed and patients delayed in getting treatment[10].

REFERENCES

1. Lei Chen, Xiaowei He, Xihong Zhao, Jianyu Su. Interaction of *Mycobacterium tuberculosis* MPB64 protein with heat shock protein 40. African Journal of Microbiology, 5(4), 2011, pp. 394-398.
2. World Health Organization. Global tuberculosis control: surveillance, planning, financing: Geneva: the Organization; 2005. WHO /HTM/TB/2005. pp.349.
3. Riccardo Manganelli,1 Eugenie Dubnau,1 Sanjay Tyagi,2 Fred Russell Kramer2, Issar Smith1 Differential expression of 10 sigma factor genes in Mycobacterium tuberculosis Molecular Microbiology, 31(2), 1999, pp. 715–724.
4. José Torres Costa, Rui Silva, Raul Sá, Maria J Cardoso and Albert Nienhaus, Results of five-year systematic screening for latent tuberculosis infection in healthcare workers in Portugal. Journal of Occupational Medicine and Toxicology, 5, 2010, pp.22.
5. Baron JE, Peterson LR, Finegold SM. Mycobacteria. In: Bailey and Scott's diagnostic microbiology. St. Louis, Missouri; Mosby. 1994, pp.590-633.

6. Iqbal S., Iqbal R., Khan M. M., Hussain I., Akhter A., Shabbir, I. Comparison of two conventional techniques used for the Diagnosis of Tuberculosis Cases. Int J Agri Biol, 5(4), 1994, pp.545-547.

7. Montenegro SH, Gilman RH, Sheen P, Cama R, Cavides L, Oberhelman RA. Improved detection of M. tuberculosis in Peruvian children by use of heminested IS6110 PCR assay. ClinInfec Dis, 36, 2003, pp.16-23.

8. Sajjad Iqbal, Dawood Amin, Asim Mumtaz and Iffat Shabir .Significance of appropriate sampling in the diagnosis of tuberculosis (TB) – A comparison of different techniques. Biomedica Vol.26, Jan. – Jun. 2010/Bio-15. pp. 39 – 44.

9. Suitters BT, Brogger SA. Some aspects of laboratory investigations in a mass campaign against tuberculosis. Bull. World Hlth Orgn, 36, 1967, pp.837-45.

10. Chakravorty S, Kamal M, Tyagi JS. Diagnosis of Extra-pulmonary tuberculosis by Smear, Culture and PCR, Using Universal Sample Processing Technology. JCM 43 (9): 2005; pp.4357-4362.

About Corresponding Author: Mr. Amit Kumar

Mr. Amit Kumar is post graduated from Himachal Pradesh University, Shimla, H.P. At post graduation level taken specialization in Medical Microbiology & Food Microbiology, and did master thesis in 'Maintenance of contamination free conditions at fruit processing plant'. In PhD taken specialization in Medical Mycology. Presently working as Assistant Professor at SILB Solan, Himachal Pradesh and pursuing PhD from Shoolini University of Biotechnology and Management Sciences, Solan Himachal Pradesh, India.